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IS 12451 (1988): Margarine [FAD 13: Oils and Oilseeds]

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IS : 12451 - 1988
(Reaffirmed 1992)

Indian Standard

SPECIFICATION FOR MARGARINE

(First Reprint SEPTEMBER 1993)

UDC 664·315·6

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

Gr 6 *March* 1989

AMENDMENT NO. 1 AUGUST 1993
TO
IS 12451 : 1988 SPECIFICATION FOR MARGARINE

(*Page* 1, *clause* **0.4**) — Substitute the following for the existing clause:

'**0.4** In the preparation of this standard, due consideration has been given to the provisions of the Prevention of Food Adulteration Act, 1954'.

(*Page* 1, *clause* **3.4.1**) — Substitute the following new clause for the existing:

'**3.4.1** Margarine may contain annato or carotene as colouring matter and diacetyl, up to a maximum of 4.0 ppm, as flavouring agent, provided that such coloured and flavoured margarine also contains starch up to a limit of 100-150 ppm. This is indicated by a blue colour using iodine solution.

NOTE — The coloured and flavoured margarine shall be sold in sealed packages not weighing more than 500 g.'

[*Page* 2, *Table* 1, *Sl No.* (iv), *col* 3] — Substitute '1.5' *for* '2.0'.

[*Page* 2, *Table* 1, *Sl No.* (vii), *col* 2] — Substitute '*Max*' for '*Min*'.

(FAD 44)

Reprography Unit, BIS, New Delhi, India

AMENDMENT NO. 2 SEPTEMBER 1996
TO
IS 12451 : 1988 SPECIFICATION FOR MARGARINE

(*Page* 1, *clause* 3.2) — Substitute the following for the existing:

"3.2 The material shall contain such quantity of refined sesame oil (*see* IS 547 : 1968†) which is sufficient to ensure that when the material is mixed with refined groundnut oil in the proportion of 20 : 80, the red colour produced by the Baudouin test shall not be lighter than 2 red units in 1 cm cell on Lovibond scale when tested as prescribed in Appendix A of IS 10633 : 1986 'Specification for *vanaspati* (*first revision*)' ."

(*Page* 1, *clause* 3.3.1) — Substitute the following for the existing:

'3.3.1 It may contain common salt, skimmed milk powder, colouring matter and flavouring agent in accordance with the provisions made in PFA Act.'

(*Page* 2, *clause* 3.6, *last line*) — Substitute '(*see* IS 14349 : 1996*)' *for* '(*see* IS : 2491 - 1972*)'.

(*Page* 2, *foot-note marked* '*') — Substitute 'Code for hygienic conditions for edible oil and *vanaspati* manufacturing units' for the existing title.

[*Page* 2, *Table* 1, *Sl No.* (vi), *col* 2] — Substitute 'at the time of sale' *for* 'when packed'.

(*Page* 5, *clause* A-1.1, *line* 2) — Substitute '(*see* IS 1070 : 1992*)' *for* '(*see* IS : 1070 - 1977*)'.

(*Page* 5, *foot-note marked* '*') — Substitute 'Reagent grade water (*third revision*)' for the existing title.

(*Page* 5, *clause* A-2.1, *line* 1) — Insert the following between the words 'sand' and 'in':

'(Quartz sand or sea sand which passes through a sieve with 10 openings per cm but not through a sieve with 40 openings per cm; if necessary, wash with hot water concentrated hydrochloric acid and water, dry and ignite at 600°C).'

(FAD 44)

Reprography Unit, BIS, New Delhi, India

Indian Standard

SPECIFICATION FOR MARGARINE

0. FOREWORD

0.1 This Indian Standard was adopted by the Bureau of Indian Standards on 30 May 1988, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Chemical Division Council and Agricultural and Food Products Division Council.

0.2 Margarine is a semi-solid food product of plastic consistency and is utilized for table use as well as baking and cooking medium. It resembles table (creamery) butter in consistency and appearance. The increase in production and consumption of bakery products and the shortage of table butter has led to the development of margarine industry in this country.

0.3 The formulation of this standard was taken up at the instance of various consumers with a view to serve as a guide in procurement of margarine of desired quality. It is also intended to serve the industry in maintaining the right quality of margarine during production.

0.4 The specification for margarine laid down under the *Prevention of Food Adulteration Act 1954* does not provide for the addition of colours and flavours. As such these are not being specified in this standard also although views in favour of their use have been expressed from time to time. However, as and when the specification under the PFA act is amended, corresponding provision would be made in this standard.

0.5 In the preparation of this standard, considerable assistance has been derived from data supplied by Shriram Foods and Fertilizers Ltd, New Delhi; Hindustan Lever Limited, Bombay; and Recommended International Standard for Margarine; issued by the Codex Alimentarious Commission of Food and Agriculture Organization of the United Nation's World Health Organization and the assistance so derived is thankfully acknowledged.

0.6 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

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*Rules for rounding off numerical values (*revised*).

1. SCOPE

1.1 This standard prescribes the requirements and the methods of sampling and test for margarine.

2. TERMINOLOGY

2.1 For the purpose of this standard, the definitions given under IS : 11476-1985* shall apply.

3. REQUIREMENTS

3.1 Description — The material shall be clean in appearance and free from rancidity. It shall have acceptable taste and flavour.

3.2 It shall contain not less than 5·0 percent by mass of sesame oil (*see* IS : 547-1968†) but sufficient to ensure that when the separated fat (*see* **A-4.2**) is mixed with refined groundnut oil in the proportion of 20 : 80, the red colour produced by the Baudouin test shall not be lighter than 2·5 red units in 1-cm cell on Lovibond scale when tested as prescribed in **6** of IS : 548 (Part 2)-1976*.

3.3 The material shall contain not less than 75 percent by mass of other edible oils or a mixture of edible oils, any or all of which may be hydrogenated. Only prescribed oils and in such specified proportions as modified from time to time by the enforcing authorities, shall be used.

3.3.1 It may be prepared with or without the addition of edible common salt and skimmed milk or buttermilk, if permitted by the competent authority.

3.4 It may also contain permitted emulsifying and stabilizing agents and butylated hydroxy anisole (BHA) and tertiary-butyl hydroquinone (TBHQ) up to a maximum limit of 0·02 percent when tested by the methods given in **A-7** and **A-8**. It may also contain vitamin D, E and theiresters or other vitamins.

3.5 The material shall be free from mineral oil and animal fat when tested by the methods as prescribed in **12** and **18** of IS : 548 (Part 1)-1964†.

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*Glossary of terms relating to oils and fats.

†Specification for sesame oil (*second revision*).

*Methods of sampling and test for oils and fats : Part 2 Purity tests (*third revision*).

†Methods of sampling and test for oils and fats : Part 1 Sampling, physical and chemical tests (*revised*).

3.6 The material shall be manufactured in the premises maintained under hygienic conditions (*see* IS : 2491-1972*).

3.7 The material shall also conform to the requirements prescribed in Table 1, when tested according to the methods prescribed in Appendix A and IS : 548 (Part 1)-1964†. Reference to relevant clauses of Appendix A and IS : 548 (Part 1)-1964† is given in col 4 and 5 of Table 1.

TABLE 1 REQUIREMENTS FOR MARGARINE

SL No.	CHARACTERISTICS	REQUIREMENT	METHOD OF TEST, REF TO	
			Appendix	Clause in IS : 548 (Part 1) - 1964*
(1)	(2)	(3)	(4)	(5)
i)	Moisture, percent by mass	12·0-16·0	A-2	—
ii)	Vegetable oils, percent by mass, *Min*	80	A-3	—
iii)	Acid value† of component vegetable oils, *Max*	0·5	—	7
iv)	Unsaponifiable matter of extracted fat‡, percent by mass, *Max*	2·0	—	8
v)	Melting point of extracted fat, °C	31-41	A-4	—
vi)	Synthetic vitamin A, expressed in international units (IU) per gram, when packed, *Min*	30	A-5	—
vii)	Salt (when added), percent by mass, *Min*	2·5	A-6	—

*Methods of sampling and test for oils and fats : Part 1 Sampling, physical and chemical tests (*revised*).

†The corresponding figure in terms of free fatty acids (FFA) when expressed as oleic acid, shall be 0·25 percent, *Max* by mass.

‡The fat shall be extracted as prescribed under A-4.2.

4. PACKING

4.1 Margarine shall be supplied suitably wrapped or packed as notified by the competent authorities from time to time.

5. MARKING

5.1 The containers shall be marked with the following:

a) Name of the material;

b) Net mass of the material;

c) Manufacturer's name and his recognized trade-mark, if any;

d) Batch No. or lot No. in code or otherwise; and

e) Month and year of manufacture.

5.1.1 The packages may also be marked with the Standard Mark.

NOTE — The use of the Standard Mark is governed by the provisions of the Bureau of Indian Standards Act, 1986 and the Rules and Regulations made thereunder. The Standard Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well defined system of inspection, testing and quality control which is devised and supervised by BIS and operated by the producer. Standard marked products are also continuously checked by BIS for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6. SAMPLING

6.0 General — Sampling requires the most careful attention to details if the subsequent analysis is to be of value. It is, therefore, essential that a truly representative sample is drawn. But this is a difficult task and it becomes more difficult when a consignment consists of a large number of packages. However, for the purpose, usual information given in the documents and certificates accompanying the consignment may be utilized to serve as a guide, and the method given in this standard should be adhered to, wherever practicable. If modification is desirable, the laboratory should be consulted regarding the selection of samples.

6.1 General Requirements

6.1.1 Samples shall be drawn by an experienced person in a protected place not exposed to damp air, bright light, dust or soot. The material shall preferably be at a temperature between 0 and 15°C at the time of drawing the sample.

6.1.2 The sampling instruments (*see* **6.2**) shall be clean and dry, and shall not impart any foreign odour or flavour.

6.1.3 Samples shall be placed in clean, odourless and dry glass containers (*see* **6.3**).

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*Code for hygienic conditions for food processing units (*first revision*).

†Methods of sampling, and test for oils and fast; Part 1 Sampling, physical and chemical tests (*revised*)

6.1.4 Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers from adventitious contamination.

6.1.5 Each container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name and address of manufacturer, and other important particulars of the consignment.

6.1.6 Samples shall be sent to the examining laboratory as quickly as possible and shall be protected from light and contaminating odours. The samples shall be stored suitably at a temperature between 0 and 5°C. No preservative shall be added to the margarine at the time of sampling.

6.2 Sampling Appliances

6.2.1 Butter triers shall be used for drawing the samples. A butter trier shall have at least 30 mm diameter and sufficient length to pass diagonally to the base of the container. The butter triers may conform to the dimensions given in Fig. 1.

6.2.2 The details of construction of the trier may be as given below.

6.2.2.1 The blade and stem of the trier should be made of stainless steel of appropriate hardness. The grip may be of stainless steel or any other suitable material which would withstand repeated sterilization.

6.2.2.2 The blade and stem may be in one piece and the transition from stem to blade shall be smooth.

6.2.2.3 The stem may be circular in cross-section and run parallel to the blade.

6.2.2.4 The blade may be tapering to the point. The degree of tapering may be less in the case of blades of triers intended for structural examination.

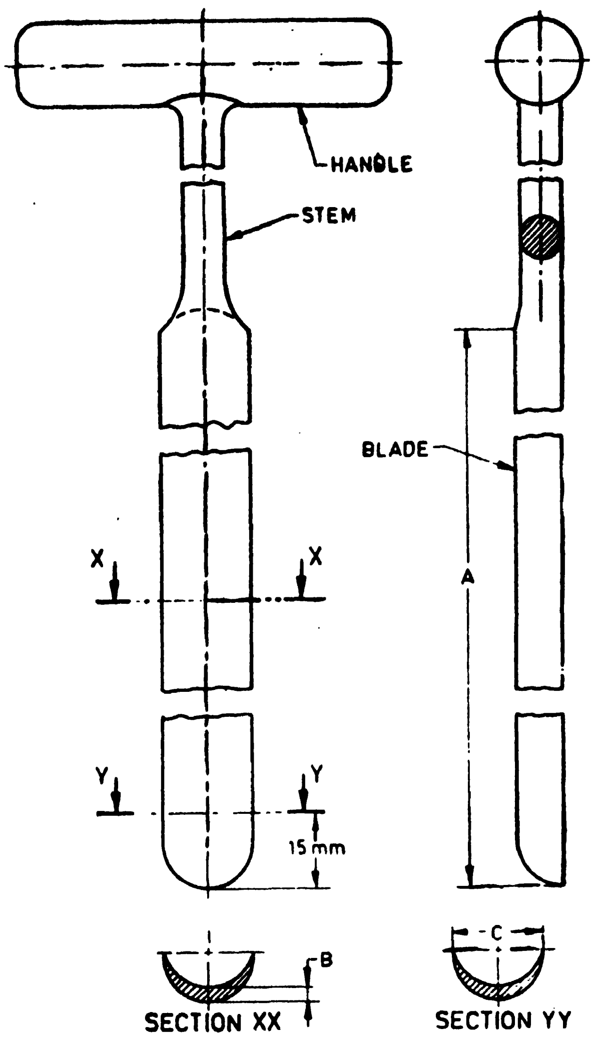
6.2.2.5 The grooves of the blade may have sufficient depth and the edges of the blade may be sufficiently sharp so as to facilitate the sampling of hard margarine.

6.2.2.6 The surface of the blade should be well polished.

6.2.2.7 Shape, material and the finish of the trier should permit the trier to be easily cleaned and sterilized.

6.2.3 Spatulas and knives may be used for removing portions of samples from the triers and should be made of stainless steel.

6.3 Sample Containers



All dimensions in millimetres.

Particulars	Principal Dimensions		
	Long	Medium	Short
*A**	540	265	125
B	1·8	1·5	0·5
C	16	16	11·5

*Tolerance for *A* is ±10 percent.

FIG. 1 BUTTER TRIER

6.3.1 Wide-mouth jars and bottles of 50 and 100-ml capacity shall be used as the sample containers. The approximate dimensions of the containers may be as given in Table 2.

TABLE 2 DIMENSIONS OF SAMPLE CONTAINERS

Sl No.	Nominal Capacity ml	Height mm	Width mm	Width of the Mouth mm
(1)	(2)	(3)	(4)	(5)
i)	50	60	50	30
ii)	100	70	60	45
iii)	200 to 250	100	70	60

6.3.2 The jar or bottle may be closed by means of a screw cap lined with butter paper or a glass stopper. Sample containers for chemical analysis may also be closed with rubber stoppers lined with butter paper.

6.3.3 Jars, bottles, bottle caps and stoppers should be suitable for sterilization.

6.4 Scale of Sampling

6.4.1 *Lot* — All the units in a single consignment belonging to the same batch of manufacture shall be grouped together to constitute a lot. If a consignment is declared to consist of different batches of manufacture, the batches shall be marked separately and the group of units in each batch shall constitute a separate lot.

6.4.2 If the margarine is supplied in bulk units like casks or boxes, the number of units to be selected for sampling shall depend on the size of the lot and shall be in accordance with Table 3.

TABLE 3 NUMBER OF BULK UNITS TO BE SELECTED FOR SAMPLING

Number of Bulk Units in the Lot N (1)	Number of Units to be Selected n (2)
2 to 9	1
10 to 49	2
50 to 99	4
100 to 199	5
Over 200	5 for the first 200 and 1 each for each 200 additional units or fraction thereof

6.4.3 If the lot is of small units such as packets or tins having the same batch number, the number of units selected for sampling shall be in accordance with Table 4.

TABLE 4 NUMBER OF SMALL UNITS TO BE SELECTED FOR SAMPLING

Number of Small Units in the Lot N (1)	Number of Units to be Selected n (2)
1 to 100	1
101 to 1 000	2
1 001 to 10 000	4
Over 10 000	4 for the first 10 000 and 1 each for 2 500 addional units or fraction thereof

6.4.4 The units to be selected for sampling shall be selected at random from the lot. To ensure randomness of selection, a random number table as agreed to between the purchaser and the supplier shall be used. In case such a table is not available, the following procedure may be adopted:

Starting from any unit in the lot, count them as 1, 2, 3,...,up to r and so on, in one order, where r is equal to the integral part of N/n, where N is the total number of units in the lot and n the number of units to be selected. Every rth unit thus counted shall be withdrawn to give the required number of units in the sample.

6.5 Sampling Technique

6.5.1 *Hard and Semi-hard Margarine Kept Under Cold Storage*

a) *From churns* — Four cores shall be drawn with the help of a trier at equal distances. At least two should be near the centre of the churn.

b) *From trollies* — Four cores (one each from the two ends and the other two from the sides) shall be drawn with the help of a trier.

c) *From boxes* — Three cores shall be drawn by inserting a trier vertically through the block. One core would be at the centre and the other two near diagonally opposite corners of the open end.

d) *From casks* — Three cores shall be drawn by inserting a trier at three points equidistant from the circumference of one end of the block and directed through the centre of the block.

e) *From small packets* — The samples shall consist of unopened packets and shall be used for chemical analysis.

6.5.2 *Pasty Margarine Kept Under Warm Conditions*

a) When the product is in small quantities, remove a sample from the deeper layers of the product at the centre of the block and two other points roughly equidistant from the central point, located 2 to 3 cm away from the ends. A suitable, clean, dry spoon, spatula or a trier should be used.

b) When the product is in the form of large heaps or blocks, select three points, one at the centre, the second about 2 to 3 cm away from the bottom and the third at an equal distance from the centre on the opposite side. At each point, draw from the deep layers three cores, roughly equidistant on the circumference. A suitable clean, dry spoon, spatula or a trier should be used.

6.5.3 *Preparation of Composite Sample* — Taking equal amount of margarine from each of the containers selected in 6.4.2 or 6.4.3, collect about 600 g or more of the material which shall be mixed and divided into three equal parts. Each part shall be transferred to a separate sample container. One of each composite samples shall be for the purchaser, the supplier and for the referee.

6.5.4 *Preparation of Sample for Chemical Analysis*

a) *Sample for analysis of margarine* — Warm the sample (*see* **6.5.1**) in an air-tight container with the lid screwed down tightly or with the glass stopper in an oven or water-bath not exceeding 40°C until by frequent vigorous shakings a homogeneous fluid emulsion (free from unsoftened pieces) is obtained at the lowest possible temperature.

b) *Sample for analysis of component vegetable oils* — Heat a portion of emulsified margarine in a beaker to a temperature of 50 to 60°C until the fat separates. Filter the fat layer through a dried filter paper into a dry vessel at a temperature above the solidification point of the fat using a hot-water funnel. If necessary, re-filter the filtrate under same conditions, until it is clean and free from water. Liquefy the fat completely and mix before taking samples for analysis.

NOTE — Exposure to light and air of the margarine sample or the vegetable oil obtained from it shall be as short as possible and analysis shall be carried out without delay.

APPENDIX A

(*Clause* 3.7)

METHODS OF TEST FOR MARGARINE

A-1. QUALITY OF REAGENTS

A-1.1 Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1977*) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

A-2. DETERMINATION OF MOISTURE

A-2.1 Procedure — Place about 20 g ignited sand in a dish provided with a glass stirring rod and weigh. Weigh about 5 g margarine to the nearest mg into the dish and dry for 1 hour in a drying oven at 105°C. Cool in a desiccator and weigh. Replace for a further half an hour in the drying oven at 105°C, cool in the desiccator and weigh again. Repeat this treatment until the mass becomes constant, that is, maximum change in mass of 0·1 percent. Keep the residue for test in A-3.

A-2.2 Calculation

Moisture, percent by mass

$$= \frac{(M_1 - M_2)}{M_1} \times 100$$

where

M_1 = mass in g of the sample, and

M_2 = mass in g of the residue.

A-3. DETERMINATION OF VEGETABLE OILS/FAT

A-3.1 Reagent

A-3.1.1 *Diethyl Ether*

A-3.2 Apparatus

A-3.2.1 *Sintered Glass Filter Crucible* — Maximum pore size 15 to 40 µm, capacity 30 to 40 mm.

A-3.2.2 *Round Bottom Flask* — 250-ml.

A-3.3 Procedure — Wash the residue after determination of moisture as in **A-2** ith 7 × 20 ml and then 4 × 25 ml diethyl ether through a weighed filter crucible and collect the filtrate in a weighed round bottom flask. Distil off ether from the filtrate in the round bottom flask. Dry the residue to constant mass in the oven at 105°C, that is maximum change in mass of 0·1 percent.

A-3.4 Calculation

Oil content, percent by mass

$$= \frac{(M_3 - M_2)\ 100}{M_1}$$

where

M_1 = mass in g of sample taken for the test in **A-2.1**,

M_2 = mass in g of the round bottom flask, and

M_3 = mass in g of the round bottom flask with residue.

A-4. DETERMINATION OF MELTING POINT OF EXTRACTED FAT

A-4.1 Apparatus

A-4.1.1 *Melting Point Tubes* — Thin-walled,

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*Specification for water for general laboratory use (*second revision*).

uniformly bored capillary glass tubes, open at both ends and with the following dimensions:

a) *Length* — 50 to 60 mm,

b) *Inside diameter* — 0·8 to 1·1 mm, and

c) *Outside diameter* — 1·2 to 1·5 mm.

A-4.1.2 *Thermometer* — Centigrade, with 0·2° subdivisions and a suitable range. The thermometers should be checked against a standard thermometer which has been calibrated and certified by the National Physical Laboratory, New Delhi.

A-4.1.3 *Beaker* — with a side-tube heating arrangement.

A-4.1.4 *Separating Funnel* — 500-ml capacity.

A-4.1.5 *Electrical Water-Bath* — with a temperature control device.

A-4.1.6 *Air-Oven*

A-4.2 Extraction of Fat

A-4.2.1 Take about 100 g of sample in a beaker. Place the beaker on water-bath maintained at 45 ± 5°C to melt the sample completely. Transfer the melted sample into a separating funnel and allow to settle for a period till water and fat layers have completely separated. Separate the water layer and dry the fat at 40-50°C under reduced pressure till constant mass.

NOTE — Care should be taken to ensure that the fat remains in liquid condition till it is separated from aqueous layer completely. This may be achieved by placing the separating funnel in an air-oven at around 50°C.

A-4.3 Procedure

A-4.3.1 Melt the sample of margarine completely and mix it well at a temperature of about 50°C. Insert the melting point tube (thoroughly cleaned and dried before use) into the molten product so that a column of the product about 10 mm long is forced into it. Allow the sample in the tube to just set, by keeping the tube in a horizontal position during winter; during summer the tube may be put on a perforated metal tray which is so placed inside the water-bath (at 15-17°C) that the bottom of the tray just touches the water. Then place the tube in a test tube immersed in water at 15-17°C for one hour. Remove the melting point tube and attach it with a rubber band or any other suitable means to the thermometer so that the lower end of the melting point tube is even with the bottom of the bulb of the thermometer. Pour water at about 20°C into the beaker (with side-tube heating arrangement) and suspend the thermometer in the centre of the beaker so that the lower end of the sample column is 30 mm below the surface of water. Heat the side-tube of the apparatus gently so that the temperature of water increases slowly at the rate of 2°C per minute till the temperature reaches 30°C and thereafter at the rate of 0·5°C per minute. Note the temperature of water when the sample column commences to rise in the tube, which is the melting point of the sample.

A-5. DETERMINATION OF VITAMIN A

A-5.1 Principle — The content of Vitamin A in margarine is determined by the measurement of ultra-violet absorption spectrum of a fraction, in which the vitamin A alcohol is collected after its isolation by chromatography. It is expressed in International Units per gram.

A-5.1.1 One International Unit (IU) of vitamin A is equivalent to 0·3 μg vitamin A alcohol or 0·344 μg vitamin A acetate.

NOTE — The official method in use in the United Kingdom (UK) involves a somewhat different procedure to that given in this method but both techniques give the same result for vitamin A in margarine.

A-5.2 Reagents

A-5.2.1 *Potassium Hydroxide* — 50 percent aqueous solution.

A-5.2.2 *Ethanol* — 96 percent (*v/v*).

A-5.2.3 *Diethyl Ether* — peroxide-free.

NOTE — Make the diethyl ether peroxide-free by distillation over potassium hydroxide (KOH). Store the peroxide-free ether over coarse granular carbon.

A-5.2.4 *Petroleum Ether* — Distilled over potassium hydroxide KOH; boiling range 40 to 60°C.

A-5.2.5 *Alumina (Activated)* — Heat the alumina at 600°C for 6 hours, cool, sieve through a 180 mesh sieve and add about 3 percent water. Mix thoroughly and allow the product to stand for at least 12 hours before use. Store in an air-tight bottle.

A-5.2.6 *Alumina (Alkaline)* — The same alumina as given in A-5.2.5 is treated with sodium hydrodixe as follows:

Stir 10 g alumina with a solution of 1 g sodium hydroxide in 10 ml water. Allow to stand at room temperature for 1 hour in a closed bottle and shake occasionally. Then heat in a dish in a vacuum drying oven at [illegible] mm Hg for 2½ hours. Pour the dried product into a bottle without removing any powder clinging to the wall of the dish and stopper securely. To the cooled powder, add 2 percent water and mix thoroughly. Allow to stand for 18 hours, and determine the moisture content after drying for 1 hour in a drying oven at 105°C; if lower than 2 percent, again add water, mix and repeat.

A-5.2.7 *Antimony Trichloride Solution* — With a porcelain spoon, introduce 65 g antimony trichloride into a 500-ml conical flask and wash several times with 15 ml chloroform until the chloroform remains clear. Then dissolve the antimony trichloride in 200 ml chloroform by refluxing. Transfer the warm solution to a bottle containing anhydrous sodium sulphate (*see* Note).

After some days, antimony trichloride crystals form at the bottom and the wall, the solution is then quite clear and ready for use.

NOTE — The chloroform to be used should be washed and dried with water before use, to remove any alcohols which may have been added to improve keeping quality.

A-5.3 Apparatus

A-5.3.1 *Conical Flask* — 50-ml.

A-5.3.2 *Separating Funnels* — 500-ml.

A-5.3.3 *Round Bottom Flask* — 200-ml with ground glass stopper and two condensers (*see* Fig. 2 and 3).

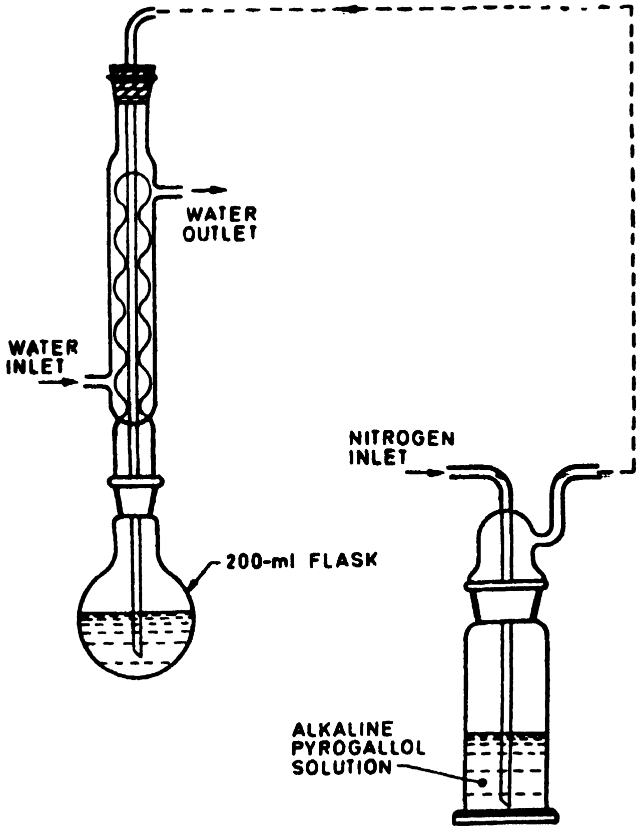


FIG. 2 APPARATUS FOR SAPONIFICATION

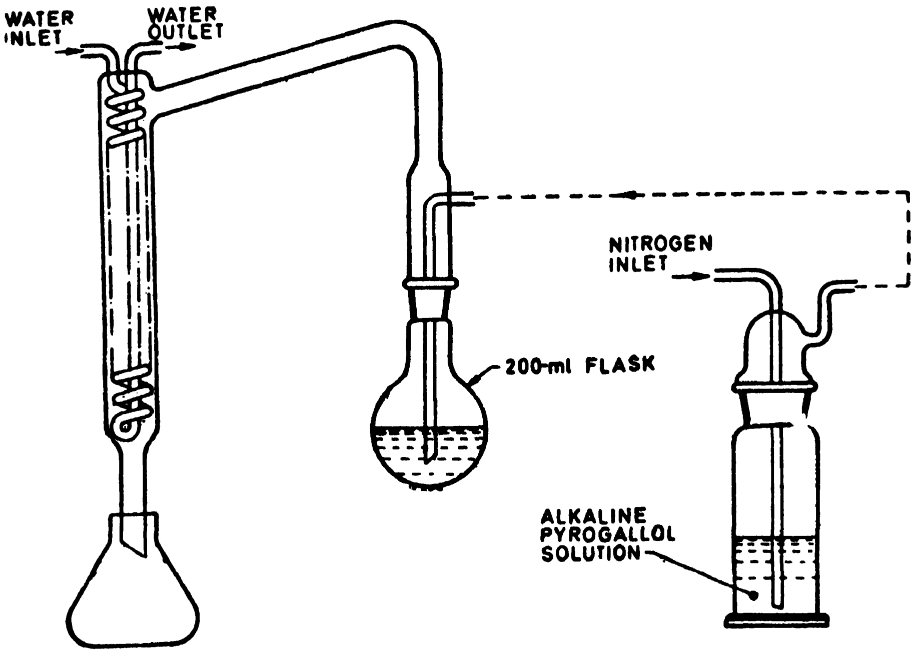


FIG. 3 APPARATUS FOR EVAPORATION

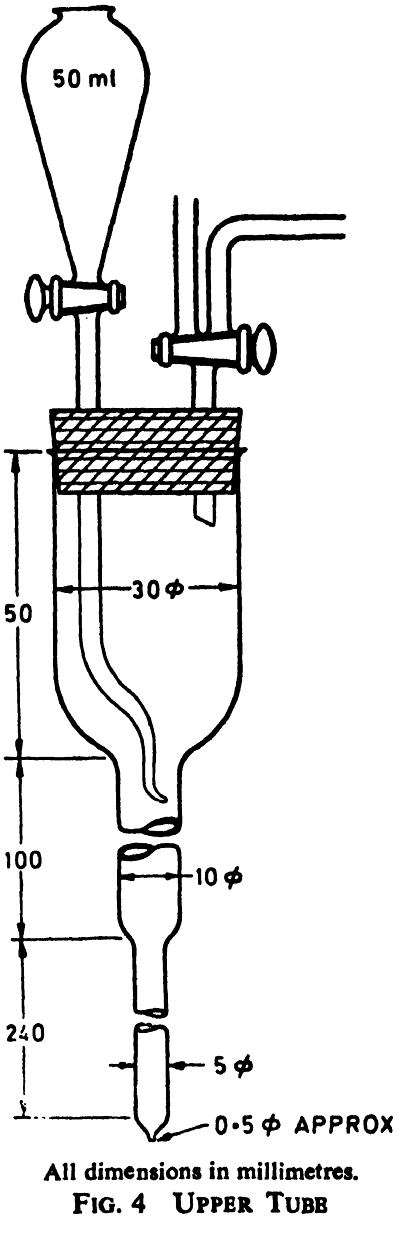
A-5.3.4 *Chromatographic Apparatus* (Fig. 4 to 7) — The apparatus consists of two parts, each containing a chromatographic column which may readily be connected in series by means of a rubber stopper connection. The upper tube contains alumina while the lower contains alkaline alumina. Close both tubes at the bottom with a plug of cotton wool. Fill the longer tube with petroleum ether to a level which reaches into the widened section, and then very regularly and gently pour out the alumina to a column height of 15 cm. Fill the shorter column, shortly before use, in the same way to a height of 2 cm with alkaline alumina.

A-5.3.5 *Tubes* — calibrated at 1 ml (Fig. 5).

A-5.3.6 *Pipette* — 1 ml with a fine tip.

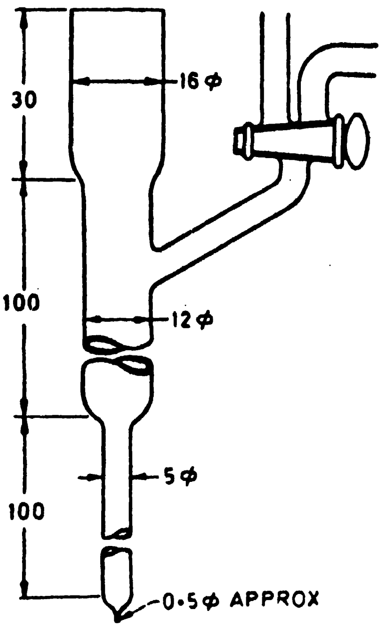
A-5.3.7 *Graduated Flask* — 10-ml.

A-5.3.8 *Ultra-Violet Spectrophotometer*



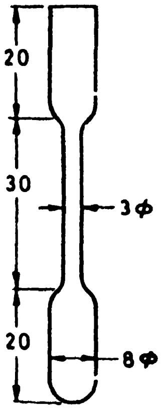
All dimensions in millimetres.

FIG. 4 UPPER TUBE



All dimensions in millimetres.

FIG. 5 LOWER TUBE



All dimensions in millimetres.

FIG. 6 RECEIVING TUBE

A-5.4 Procedure

A-5.4.1 *Sampling* — Cut from a block of margarine, a slice 2 to 3 cm thick and from this, cut off a slice weighing approximately 10 g. Such a sample contains inside as well as outside parts of the margarine, and should contain a total of about 250 IU of Vitamin A.

A-5.4.2 *Saponification of Margarine* — Put the slice into 200-ml round bottom flask and weigh to the nearest 50 mg. Add to the margarine, 8 ml of potassium hydroxide solution and 25 ml of ethanol. Heat gently on the water-bath at 85 to 90°C for a quarter of an hour or until the saponification is complete, with a reflux condenser attached to the flask. During this procedure, a slow current of oxygen-free nitrogen is passed through the liquid.

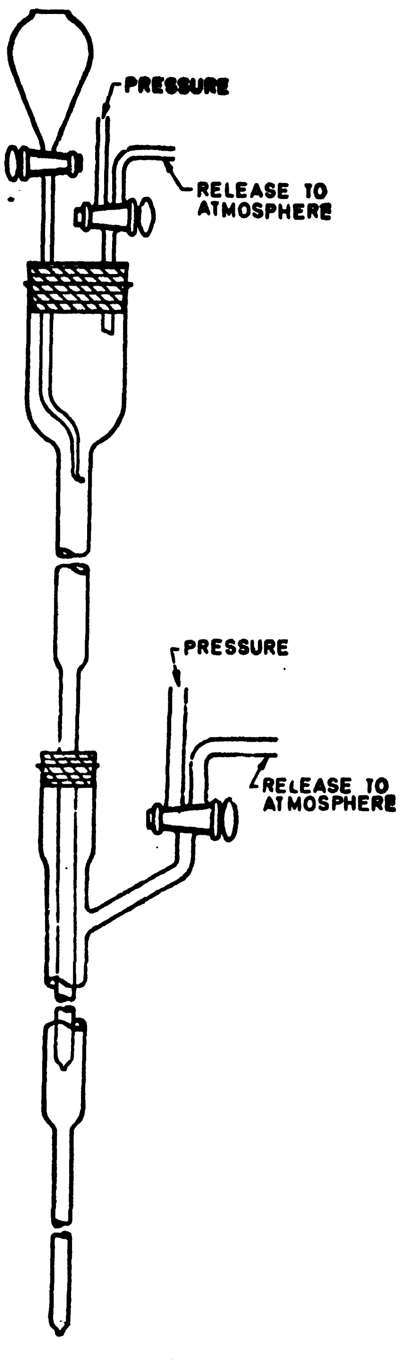


FIG. 7 ASSEMBLY OF CHROMATOGRAPHIC TUBES

A-5.4.3 *Extraction of Vitamin A* — Add 50 ml water through the condenser and cool the soap solution in tap water. Transfer the solution to a separating funnel, using another 50 ml water to rinse the flask. Extract the soap solution with successive portions of 100, 50, 50 and 50 ml diethyl ether by shaking. Wash the combined extracts in another separating funnel four times with 50 ml of water (the first time only by swirling, the following three times by gentle shaking). Continue washing if the ether layer is still turbid.

A-5.4.4 *Evaporation of Solvent* — Use the same flask in which the saponification was carried out. Add diethyl ether solution in two portions. Heat on the water-bath (80 to 85°C). During the distillation, maintain a weak current of oxygen-free nitrogen until approximately 5 ml is left. Then, transfer the residue to a 50 ml conical flask, and rinse with 15 to 20 ml ether. Evaporate the ether with nitrogen, add some acetone, and again evaporate the solvent.

Immediately take up the residue in 1 to 2 ml petroleum ether (if not clear, dry again with acetone), and introduce the solution on top of the upper chromatographic column with as little petroleum ether as possible (about 5 ml).

A-5.4.5 *Chromatographic Separation of Vitamin A Alcohol* — The chromatographic separation is carried out in two stages. For the first stage, only the upper column is used (alumina). After the petroleum ether extract has been brought on to the column, this is passed through the adsorbent. Rinse the conical flask with 5 ml petroleum ether and bring this amount on to the column. Rinse the flask with 5 ml portions of petroleum ether containing 4, 8 and 12 percent diethyl ether (*v/v*) respectively, and use the washings for elution process. Discard all these elutes containing substances which are less strongly adsorbed than Vitamin A alcohol.

NOTE — Test for Vitamin A with antimony trichloride in the elute containing 12 percent diethyl ether. If the reaction is positive (in which case the alumina contains too much water), repeat the determination with a fresh column packing.

A-5.4.6 Then connect the second column (alkaline alumina) and elute with 5 ml each of petroleum ether containing 16, 20 and 24 percent diethyl ether (*v/v*), respectively. Finally, use petroleum ether containing 36 percent diethyl ether (*v/v*) until the Vitamin A has been eluted completely (*see* Note). Collect the eluted fractions in the tubes graduated at 1 ml. First thoroughly mix the contents of each tube in order to obtain a homogeneous solution. Do this by blowing some air bubbles through by means of the fine-tipped pipette. Then with the same pipette, remove from each of the tubes approximately 0·3 ml and with these samples carry out the Carr-Price spot test (use a small test tube, 0·5 ml antimony trichloride solution, and 1 drop of acetic anhydride).

NOTE — Ensure that during elution, the columns do not run dry, but at the same time prevent, as much as possible, the various petroleum ether fractions from mixing at the top of the first column. Only very little elute originating from the first column may be present on top of the second column.

A-5.4.6.1 Discard the fractions in which the Carr-Price spot test is negative [generally those with 16 and 20 percent diethyl ether (*v/v*)].

NOTE — It is recommended to occasionally compare the shape of the optical density curve between 260 and 370 µm with Morton-Stubb's ideal curve. This is done to establish whether the separating capacity of the columns is sufficient. Thus, freshly prepared adsorbents should always be tested in this way.

A-5.4.7 *Spectrophotometric Determination of Vitamin A* — Pipette exactly 0·5 ml from each tube in which the Carr-Price spot test is positive. Pool these in the 10 ml graduated flask, make up to the mark with petroleum ether used and mix. Make sure that the absorption at 326 μm of the petroleum ether used does not change when 10 percent diethyl ether is added. Use petroleum ether in the blank cell. Read the optical density at the top of the extinction curve (324 to 326 μm) using a 1 cm cell (*see* Note).

NOTE — It is recommended to occasionally compare the shape of the optical density curve between 260 and 370 μm with Morton-Stubb's ideal curve. This is done to establish whether the separating capacity of the columns is sufficient. The freshly prepared adsorbents should always be tested in this way.

A-5.5 Calculation

$$\text{Vitamin A (I U/g)} = \frac{366\,a}{M}$$

where

a = optical density reading at the maximum, and

M = mass in g of sample.

A-6. DETERMINATION OF SALT (SODIUM CHLORIDE)

A-6.1 Principle — The sodium chloride is extracted from margarine by hot water and determined by Mohr's method.

A-6.2 Reagents

A-6.2.1 *Silver Nitrate* — 0·1 N, standard solution.

A-6.2.2 *Potassium Chromate* — 10 percent aqueous solution.

A-6.3 Procedure — Weigh accurately about 5 g of the sample into a 250-ml conical flask. Carefully add 100 ml of boiling water. Allow to stand with occasional swirling for 5 to 10 minutes. After cooling to 50 to 55°C (titration temperature), add 2 ml of potassium chromate solution. Mix by swirling. Titrate at 50 to 55°C with standard silver nitrate solution while swirling continuously until the brownish colour persists for half a minute. Carry out a blank test with all the reagents in the same quantity except the sample material.

A-6.4 Calculation

Sodium chloride, percent by mass

$$= \frac{5{\cdot}85\,N\,(\,V_1 - V_2\,)}{M}$$

where

N = normality of silver nitrate solution,

V_1 = volume in ml of silver nitrate used in sample titration,

V_2 = volume in ml of silver nitrate used in the blank titration, and

M = mass in g of the sample taken.

NOTE — This method is not applicable to margarine containing sodium chloride up to 0·2 percent by mass or less.

A-7. ANALYSIS OF TERTIARY BUTYL HYDROQUINONE (TBHQ)

A-7.1 Apparatus

A-7.1.1 *Volumetric Flasks* — 50 and 100-ml.

A-7.1.2 *Pipettes* — 1, 2, 3, 4, 5 and 10-ml.

A-7.1.3 *Beakers* — 50 and 250-ml.

A-7.1.4 *Soxhlet Apparatus*

A-7.1.5 *Rotary Vacuum Evaporator*

A-7.1.6 *UV Spectrophotometer*

A-7.2 Reagents

A-7.2.1 *Methanol*

A-7.2.2 *Diethyl Ether* — anhydrous.

A-7.2.3 *Diethylamine Reagent* — 25 percent in water.

A-7.2.4 n-*Butanol*

A-7.3 Preparation of Standard Solution

A-7.3.1 *Stock Solution* — Weigh 0·1 000 g of TBHQ into a weighing boat, transfer to a 100-ml volumetric flask and make to volume with methanol. This solution contains 1 000 micrograms of TBHQ per ml.

A-7.3.1.1 Pipette 10 ml of the stock solution into a 100-ml volumetric flask and make to volume with methanol. This standard solution contains 100 μg of TBHQ.

A-7.3.2 Pipette aliquots of TBHQ standard into reagent bottles to give a range of 100 to 1 000 μg of TBHQ per aliquot. Add enough methanol to each bottle to give a total volume of 10 ml. Prepare a blank with 10 ml of methanol. Add 5 ml of dimethylamine reagent to each bottle. Stopper and shake thoroughly. Allow the solutions to stand for 30 min, then add 10 ml of *n*-butanol to each bottle and mix well. Using the blank in the reference cell, measure the absorbancies of the standards at 500 nm.

7.3.3 Calculate the individual *k* values by dividing the absorbance measurement by respective concentration of TBHQ in each standard aliquot.

A-7.4 Extraction of TBHQ

A-7.4.1 Weigh about 20 g of the extracted fat (*see* A-4.2) into a 250-ml flask. Add 50 ml of methanol and connect the flask to reflux condensers and gently boil for 1 h, apply just enough heat to liquefy the fat and cause gentle boiling. During the reflux period, occasionally shake the reflux units to ensure thorough mixing of the fat and the alcohol. Allow to cool to room temperature. Separate from the condensers, stopper and allow to stand until the contents have completely separated into two layers.

A-7.5 Analysis

A-7.5.1 Pipette 1 to 10 ml of the methanol extract (upper layer obtained in **A-7.4.1**) into reagent bottles. Add methanol to give a total volume of 10 ml. Prepare a blank of 10 ml of methanol. Add 5 ml of dimethylamine reagent to each bottle. Stopper and shake vigorously.

NOTE — The extract may become turbid on addition of dimethylamine. This turbidity normally clears on addition of *n*-butanol in **A-7.5.2**.

A-7.5.2 Allow the solutions to stand for 30 min, then add 10 ml of *n*-butanol to each bottle and mix well Using the blank in the reference cell, measure the absorbance of the sample aliquot at 500 nm.

A-7.6 Calculation

Tertiary butyl hydroquinone, percent by mass

$$= \frac{A\,V_1}{B\,V_2\,M} \times 10^{-4}$$

where

A = absorbance of the sample at 500 nm,

V_1 = volume in ml of methanol used in extraction,

B = k value for TBHQ as determined with TBHQ standard solutions,

V_2 = volume in ml of methanol extract taken for analysis, and

M = mass in g of fat analyzed.

A-8. DETERMINATION OF BUTYLATED HYDROXY ANISOLE (BHA)

A-8.0 General — Butylated hydroxy anisole is separated from the fat by distillation with superheated steam. The distillate is analyzed for butylated hydroxy anisole with 2,6 dichloroquinonechloroimide.

A-8.1 Apparatus

A-8.1.1 *Distillation Apparatus* — The distillation apparatus consists of a steam generator consisting of a 1 000-ml Erlenmeyer flask containing water and several glass beads. The rubber stopper in the flask has an outlet tube ending in a 12/5 socket joint. Water is boiled on an electric heater. The superheater consists of a glass coil placed in a 1 000-ml beaker half full of wax (melting point 60°C, smoke point 27°C, flash point 36°C), and provided with a thermometer. The distilling flask is made from a 50/50 standard-taper joint, a 12/5 and a 28/15 ball joint. The distilling flask is heated in a 1 000-ml beaker containing approximately 800 ml of bath wax. During distillation, the inlet and outlet tubes of the superheater and the distilling flask are wrapped in glass wool. The condenser is 61 centimetre long with a 28/15 socket joint. The distillate is filtered and collected in a 250-ml glass stoppered graduate.

A-8.1.2 *Photoelectric Colorimeter* — with 620 µm filter and cells

A-8.2 Reagents

A-8.2.1 *Ethyl Alcohol* — Add approximately 0·1 percent potassium hydroxide and potassium permanganate to commercial absolute alcohol and distil in all-glass apparatus. The distillate is 100 percent alcohol.

A-8.2.2 *Calcium Chloride*

A-8.2.3 *Borax Buffer* — Prepare 2 percent borax ($Na_2B_4O_7 . 10H_2O$) in water.

A-8.2.4 *n-Butyl Alcohol*

A-8.2.5 *2,6-Dichloroquinonechloroimide Reagent*

A-8.3 Distillation — Place 16 g of anhydrous calcium chloride (reagent grade) and 10 ml of distilled water in the distilling flask; cool to approximately room temperature and weigh 5 g of the sample in the flask. Lightly grease the round-glass joint and place the top on the distilling flask.

A-8.3.1 Before starting the distillation, heat the bath for the distilling flask to 160 ± 10°C, and the superheater bath to 200 ± 20°C, adjust the steam generator to distil approximately 4 ml of water per minute. Maintain these conditions throughout the entire distillation.

A-8.3.2 As soon as these conditions are fulfilled, connect the superheater and the condenser to the distilling flask. Start the distillation by connecting the steam generator to the superheater and immediately place the bath around the distilling flask. Collect the distillate in a 250-ml glass-stoppered graduate, filtering the distillate through a 9-cm Whatman filter paper No. 54 or equivalent, as it collects. The rate of distillation shall be such that 125 ml of distillate collects in 30 ± 5 minutes.

A-8.3.3 When 125 ml of distillate has been collected, stop the distillation by disconnecting the distilling flask from the superheater and removing the bath around the distilling flask. When the mouth of the condenser has cooled, disconnect it from the distilling flask and drain the water from the water jacket. Wash the condenser and filter paper thoroughly, using six 10-ml portions of hot (60 ± 5°C) 100 percent ethyl alcohol, allowing the alcoholic washings to filter into the distillate. Continue washing the filter with hot alcohol until the combined volume of distillate and washings is 250 ml when cooled to room temperature.

A-8.4 Analysis of Distillate — Pipette three different aliquots of the distillate (50 percent alcohol) into 19-mm colorimeter tubes and make up to 12 ml with 50 percent ethyl alcohol. Add 2 ml of the 2, 6-dichloroquinonechloroimide reagent and 2 ml of borax buffer and mix. After 15 minutes, add 5 ml of *n*-butyl alcohol to each tube, mix, and measure the absorbancy with a photoelectric colorimeter using a 620 µm filter. Measure all absorbancies relative to a reagent blank.

A-8.4.1 Prepare a reference curve over a range of 10 to 50 µg of butylated hydroxyanisole. The concentration of butylated hydroxyanisole, in microgram per aliquot used, is obtained by dividing the observed absorbancy by a *k*-value of 0·010 2.

A-8.5 Calculation — Calculate the amount of butylated hydroxyanisole in the distillate as follows:

Concentration of BHA in margarine sample

$$= \frac{A \times V_2}{M \times V_1 \times 0.010\,2 \times 10^6} \times 100$$

where

A = absorbancy,

V_2 = total volume of distillate and washing in ml.

M = mass in g of the sample, and

V_1 = aliquot volume in ml.

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